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The acute transcriptomic response of coral-algae interactions to pH fluctuation

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ABSTRACT

Little is known about how the coral host and its endosymbiont interactions change when they are exposed to a sudden nonlinear environmental transformation, yet this is crucial to coral survival in extreme events. Here, we present a study that investigates the transcriptomic response of corals and their endosymbionts to an abrupt change in pH (pH 7.60 and 8.35). The transcriptome indicates that the endosymbiont demonstrates a synchronized downregulation in carbon acquisition and fixation processes and may result in photosynthetic dysfunction in endosymbiotic *Symbiodinium*, suggesting that the mutualistic continuum of coral–algae interactions is compromised in response to high-CO_2 exposure. Transcriptomic data also shows that corals are still capable of calcifying in response to the low pH but could experience a series of negative effects on their energy dynamics, which including protein damage, DNA repair, ion transport, cellular apoptosis, calcification acclimation and maintenance of intracellular pH homeostasis and stress tolerance to pH swing. This suggests enhanced energy costs for coral metabolic adaptation. This study provides a deeper understanding of the biological basis related to the symbiotic corals in response to extreme future climate change and environmental variability.

1. Introduction

Mutual benefits with endosymbiotic photosynthetic *Symbiodinium* are fundamental to corals thriving in nutrient-poor tropical seas (Kopp et al., 2013). Within this association, members of *Symbiodinium* receive protection from external predators while receiving access to host-excreted inorganic nutrients, namely, carbon dioxide (CO_2) and ammonium (NH_4^+) (Wooldridge, 2010). In return, the coral host acquires a vast majority of photosynthates for its carbon and energy demands. However, the details of the physiological action of metabolic interaction between symbionts and coral are still poorly understood. The dynamic balance between partners during reciprocal exploitation may confer a stronger metabolic plasticity and greater adaptive selection in response to environmental disturbances (Lesser et al., 2013). Still, the extreme events of anthropogenic-induced environmental variability are probably unprecedented and raise the crucial question of whether coral–algal mutualistic symbiotic ecosystems are destabilized by environmental stress on a short timescale. We are particularly interested in the potential effects of fluctuations in CO_2 concentrations and pH on coral-algae interactions (Wooldridge and Done, 2009). Specifically, increased CO_2 concentrations would alter the level of dissolved inorganic carbon (DIC) that is crucial for both photosynthesis and calcification (Bertucci et al., 2013). There has been limited consideration of the intimate links between the precipitation of calcium carbonate and the photophysiological regime of the endosymbiotic algae; therefore, one of the main questions that remains to be answered is how environmental changes affect this carbon flow and allocation.

Natural pH variability is relatively small in the open ocean; most studies have focused on the biologically relevant variables across contrasting biogeographic regions or in seasonal and/or diurnal variability. However, acute and extreme pH change have received little attention (Hofmann et al., 2011). In marine invertebrate organisms at the molecular level, physiological response induces dynamic epigenetic events, such as histone acetylation and phosphorylation, within one or two hours after the start of exposure to a novel environment (O'Donnell et al., 2009). Acute responses tend to tell us more information about the stressor itself than about expected long-term effects and are more relevant to the original event (Baum et al., 1990). Some coral populations are relatively resilient to environmental disturbances and show higher tolerance levels; such studies involving the acute responses of extreme events can provide hypotheses about how organisms cope with or adapt to upcoming, unpredictable/uncontrollable environmental variability. Additionally, in the current era of a rapidly changing climate, corals experience large swings in pH, both internally and on a reef scale, on a daily basis. Therefore, it is imperative to understand the mechanisms of the acute pH response and rapid shifts in cellular properties, and there

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is some merit in understanding the effects of ocean acidification on such a process (Barshis et al., 2013).

In this study, we investigate the transcriptomic response of corals and their endosymbionts to an abrupt change (duration 3 h) in pH and document the molecular mechanisms behind an extreme pH change. The magnitude of pH decrease (pH 7.6) chosen here is based on climate change projections (Caldeira and Wickett, 2005), although it is unlikely to occur in current natural seawater conditions, which could be helpful for examining the stress response of corals to environmental hypercapnia (Tremblay et al., 2013). Moreover, choosing *Galaxea fascicularis* for this study is based on our preliminary experiments; a short period (4 weeks) of mesocosm-scale seawater acidification (pH 8.0–7.6) induced initial mortality (10–25%) in the *G. fascicularis* colonies, supporting the prediction that *G. fascicularis* is a relatively tolerant species to acidification perturbation (Hii et al., 2009). It also helps to liberate their plasticity of gene expression performance. We endeavor to extend the global transcriptomic model by considering the possibility that the acute pH change-induced damage is triggered by a dysfunction in the mutualistic benefit continuum between the coral host and its endosymbiont partners, which may play an important role during the acidification process that threatens to bleach and ruin the coral reef. On the other hand, many active regulatory metabolic adaptation mechanisms are involved in energetic allocation, stress defenses and calcification compensation, which have vitalizing effects on some coral species that enable rapid acclimation to pH swings and a glimmer of hope for the survival of corals.

2. Materials and methods

2.1. Coral species and experimental setup

During July 2015, several adult colonies of the *G. fascicularis* (mt-L1 type) (Nakajima et al., 2016; Lin et al., 2017b) were collected in Hainan Island, South China Sea (E110°65′, N19°25′). The tops of the colonies were split into approximately 5 cm × 5 cm in size, using bone cutters, and then were immediately transported to the same aquaculture facilities at the Third Institute of Oceanography (TIO), State Oceanic Administration, where they were maintained in a running filtered seawater aquarium system, under controlled conditions (25 °C, pH_T 8.15, salinity of 35). The corals received constant photosynthetically active radiation (PAR) of 180 μmol m^{-2} s^{-2} (photoperiod was 12 h:12 h light/dark) using HQI-10000 K metal halide lamps (BLV-Nepturion). These coral nubbins were left to allow tissues to grow and recover for a period of two months prior to use in experiments.

For the 3-h exposure experiments, *G. fascicularis* colonies were placed into six 30-L tanks. There were two pH treatments; three tanks (control tank) were not bubbled with CO_2 at ambient pH 8.35 ± 0.1 (280 ppm CO_2), and three acidulated experimental tanks at pH 7.60 ± 0.1 (1800 ppm CO_2) were maintained by bubbling with CO_2. The concentration of CO_2 was obtained using a flow meter. After 3 h of exposure, 3 colonies in each tank were sampled at the same time and stored in liquid nitrogen until analyzed. Total alkalinity (TA) and DIC were also analyzed to describe the inorganic carbon system in the tanks. TA and DIC were significantly higher in the acidulated treatment tanks compared to the control (Table 1). pH was measured according to the National Bureau of Standards (NBS) scale, with a portable meter (ST 2100, OHAUS) calibrated daily with pH 4 and pH 7 buffers. TC, DIC and TOC were measured using a Shimadzu model TOC-L analyzer (Shimadzu Corp., Kyoto, Japan). TA was measured by open-cell potentiometric titration. HCO_3^-, CO_3^{2-}, Ω aragonite and Ω calcite were calculated by measurement of TA, DIC and salinity, using the CO_2 SYS program.

2.2. RNA extraction and high-throughput sequencing

Approximately 5 μg of total RNA (pooled in equal amounts from three biological replicates) for each tank was used for library construction. Total RNA was extracted from each colony using TRIzol (Invitrogen Life Technologies, CA, USA), according to the manufacturer's protocol, and dissolved in RNase-free water. Ten micrograms of total RNA from each coral sample was used to prepare the cDNA library using the NEBNext® Ultra™ RNA Library Prep Kit, following the manufacturer's recommendations. Sequencing was conducted on an Illumina Hiseq 2500 platform at Novogene Bio-pharm Technology Co., Ltd. (Beijing, China). Clean data were obtained by removing reads containing the adapter, reads containing ploy-N and low quality reads (Q < 20) from raw data using SeqPrep (https://github.com/jstjohn/SeqPrep). At the same time, the Q20, Q30, GC-content and sequence duplication levels of the clean data were calculated (Workbench). Cleaned reads of a length threshold of 200 bp were mapped onto the publicly available NCBI TSA of assembled transcriptome *G. fascicularis* (Lin et al., 2017b), using TopHat 1.2.0 with the '–b2-very-sensitive' option (Trapnell et al., 2009). The obtained sequences were defined as unigenes. The repetitive sequences were identified in the final gene set by searching similar sequences with a minimum similarity cut-off of 95%, using CD-HIT-EST. CD-HIT was used for further clustering with a 90% similarity cut-off (http://weizhong-lab.ucsd.edu/cd-hit/).

Table 1
Carbonate chemistry parameters measured and calculated from pH treatments, total alkalinity (TA), temperature (25 °C) and salinity (35). DIC: dissolved inorganic carbon; TC: total carbon; TOC: total organic carbon.

Measured parameters						Calculated parameters			
pH scale (pH_T)	TA (μmol/kg)	pCO_2 (μatm)	TC (mg/L)	DIC (mg/L)	TOC (mg/L)	HCO_3^- (μmol/kg)	CO_3^{2-} (μmol/kg)	ΩCa	ΩAr
8.35	2398	280	40.74	39.98	0.76	1491.74	370.08	8.91	5.87
7.60	2774	1800	47.57	47.17	0.40	2511.23	110.79	2.67	1.76

2.3. Identification of host-derived and symbiont-derived sequences

All currently available *Symbiodinium* and cnidarian transcriptomes and genomes were obtained from public databases (http://comparative.reefgenomics.org/datasets.html) (Bhattacharya et al., 2016) and assembled into two distinct databases of the coral host and *Symbiodinium* symbionts. The taxonomic assignment of sequences from the coral host or *Symbiodinium* symbiont were predicted using the approach of the top hit species from the BLASTn results, with *E*-value < 1e-5 (the species with the best/first sequence alignment for a given BLASTn result). If a sequence was present both in the host and symbiont transcriptomes after filtering, the sequences were BLASTx-searched (E-value cutoff: 1e-5) in a NCBI nr database to predict with high confidence whether they originate from animal or phytoplankton. The sequence length and GC content distribution of the coral host-derived and *Symbiodinium*-derived sequences were further investigated to evaluate the effects of identification.

2.4. Transcriptome annotation

Eukaryotic unigenes were annotated by BLAST sequence homology searches against the UniProt, Swiss-Prot, Pfam, and NCBI NR protein databases, with an e-value cutoff of e^{-5}. Gene Ontology (GO) (http://www.geneontology.org/) annotations and the corresponding enzyme commission numbers (EC) of the sequences were obtained using the Blast2GO software (http://www.blast2go.org/). Perl scripts were generated to extract the metabolic annotation data from KEGG (Kyoto Encyclopedia of Genes and Genomes) metabolic annotation files (ftp://ftp.genome.jp/pub/kegg/genes/organisms/pic/).

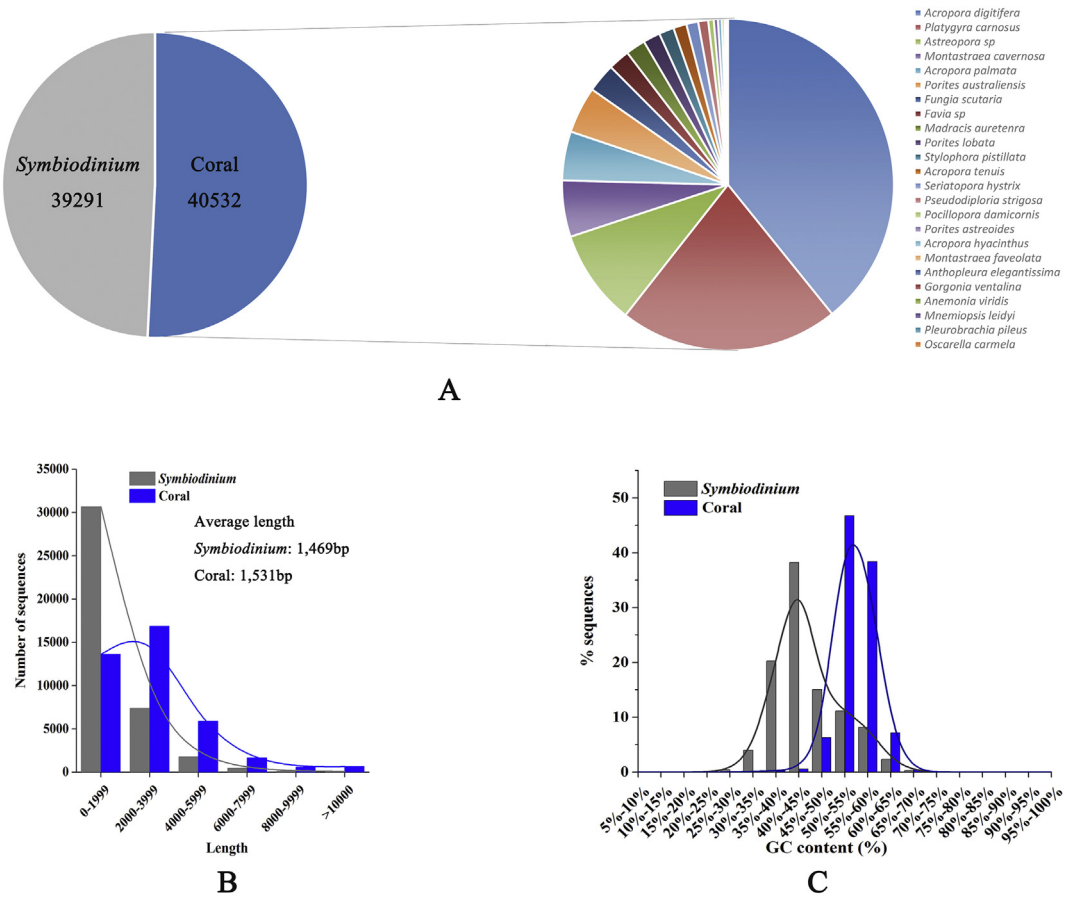

Fig. 1. Analyses of the coral host-derived and *Symbiodinium* symbiont-derived transcriptome assembly. (A) Coral species distribution of top BLAST results. (B) Length distribution of transcripts in the transcriptome assembly. (C) Distribution of GC percentages of the assembled transcripts.

2.5. Differential gene expression analysis

Gene expression levels were measured as RPKM (Reads Per kb per Million reads) as described by (Mortazavi et al., 2008). For RNA-seq-based expression analysis, RPKM is able to eliminate the influence of different gene lengths and sequencing data amounts on the calculation of gene expression. Differential gene expression analysis across groups was inferred from the RPKM, using the edge R package (Robinson et al., 2010). The identified genes with a fold change ≥ 1.0 and a false discovery rate (FDR) < 0.05 were considered as significant DEGs. Significantly enriched KEGG pathways were identified with KOBAS 2.059, using a hypergeometric test and the Benjamini-Hochberg FDR correction (Xie et al., 2011).

2.6. Quantitative PCR validation of target genes

The coral-derived and *Symbiodinium*-derived transcriptomes were used for the subsequent expression analysis. We performed RT-PCR to assess the expression levels of the coral-derived and *Symbiodinium*-derived candidate genes (Table S2), and their levels were consistent with the levels in the transcriptomic data (Fig. S1). Each RNA sample was adjusted to 1 μg/μl with nuclease-free water. Two micrograms of total RNA was reverse transcribed in a 20-μl reaction volume using the PrimeScript™ RT reagent kit with gDNA Eraser (Takara, Dalian, China). The glyceraldehyde-3-phosphate dehydrogenase (GADPH) and β-actin (ACT) genes were used as internal controls for coral and *Symbiodinium*, respectively. The qRT-PCR was performed using the SYBR® *Premix Ex Taq*™ (Tli-RNase H Plus) (Takara, Dalian, China), according to the manufacturer's protocol. The qPCR analysis was performed in triplicate for each extracted RNA sample. PCR amplifications were performed using an RG 6000 (Qiagen, Hilden, Germany) in a 20-μl reaction volume. The results were analyzed using the Rotor-Gene®Q software. Raw data were analyzed using the default settings of the software. A relative quantitative method ($^{\Delta\Delta}$Ct) was used to evaluate the quantitative variation (Livak and Schmittgen, 2001).

3. Results

3.1. Host- and symbiont-derived transcriptome composition

To analyze the transcriptome of the coral host and its endosymbiont algae partner response to a sudden exposure to pH change, we conducted RNA-seq of the pH 7.60 and pH 8.35 samples from *G. fascicularis* colonies. Subsequent quality filtering and adapter trimming yielded 167 million pair-end PE reads (Table S1), which were assembled into a reference *G. fascicularis* transcriptome containing 347,084 assembled unigenes. Of 347,084 total unigenes, 40,532 sequences were classified as coral transcripts, based on BLASTn hits to known coral databases (Shinzato et al., 2011; Bhattacharya et al., 2016); the species match was studied where nearly 40% of the unigenes showed significant homology with those of *Acropora digitifera*, and 22% showed significant similarities with those of *Platygyra carnosus* (Fig. 1A). In total, 39,291 transcripts had significant BLASTn hits to either of the known *Symbiodinium* databases. All *G. fascicularis* samples maintained *Symbiodinium* C1 as the dominant symbiont; beyond that, no other symbiont type was detected by mapping the raw sequence data to ITS2 (Lin et al., 2017a). The contrasting frequency distribution of the sequence length and GC content showed that the coral-derived sequences exhibited longer transcripts and higher GC content than the *Symbiodinium*-derived sequences (Fig. 1B–C).

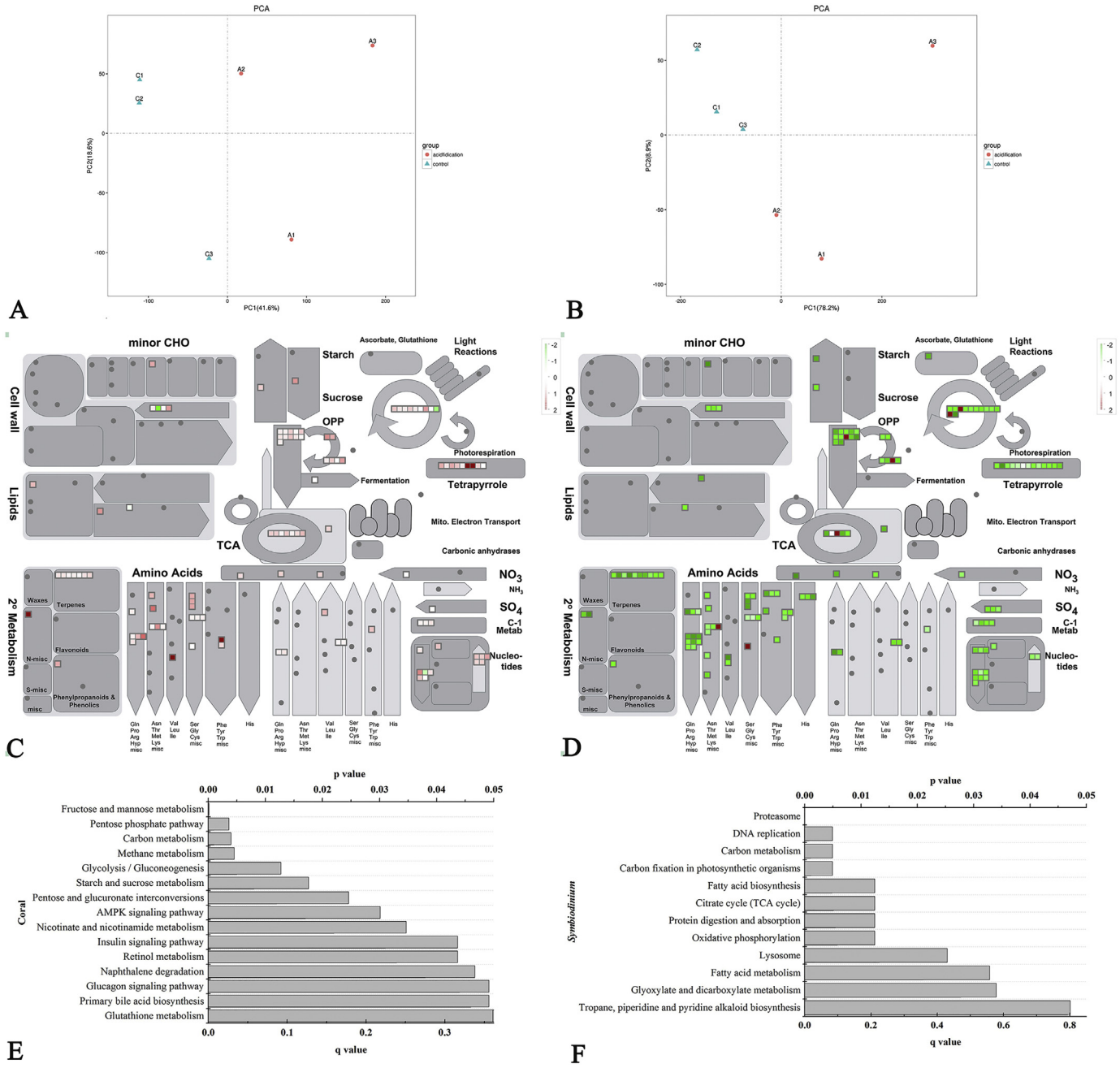

Fig. 2. Characteristics of gene expression in the host (left panels) and symbiont (right panels) from the *G. fascicularis* abrupt pH change experiment (pH 7.60 vs pH 8.35). Principal component analysis based on the expression level data (A–B). Overview of biological pathways with differential expression changes (C–D). Image is retrieved from the MapMan software (version 3.5.1R2). Green and red blocks denote downregulated and upregulated genes, respectively. The statistically overrepresented KEGG pathway categories for differentially expressed genes (E–F). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. Response of coral-algae partners to energy and nutrition store dynamics

For both host and *Symbiodinium*, acute pH treatment caused a shift in transcriptome profile, as indicated by the distinct clustering of pH 7.60 corals from pH 8.35 corals in the PCA plot (Fig. 2A–B), in which the pH treatment group was separated along the axes of PC2. The coral- and *Symbiodinium*-origin genes belonging to various functional categories are represented in Fig. 2C–D using the MapMan software. In the coral host expression profile, more genes belonging to C- and N-associated biosynthetic pathways, such as glycolysis, the TCA cycle, starch/sucrose/lipid metabolism, and amino acid synthesis, were prevalently induced under elevated CO_2, though the expression changes for many genes were relatively weak (< 2-fold). However, in the *Symbiodinium* fraction we detected reductions in the expression of transcripts linked to the aspects of central metabolism, namely, the TCA cycle, energy and amino acid metabolism. With respect to pathway enrichment analysis of differentially expressed (FDR < 0.05 and |log2-fold change| > 1.0) transcripts, showing top-ranked categories including energy metabolism and catabolism, responses to carbohydrate and sucrose stimulus, generation of energy precursor metabolites and interconversion of carbon compounds and energy-sensing pathways, which appeared significantly over-represented at the onset of the acidification stress induced by CO_2 elevation in two common hosts and symbionts (Fig. 2E–F). These results suggested that the maintenance of

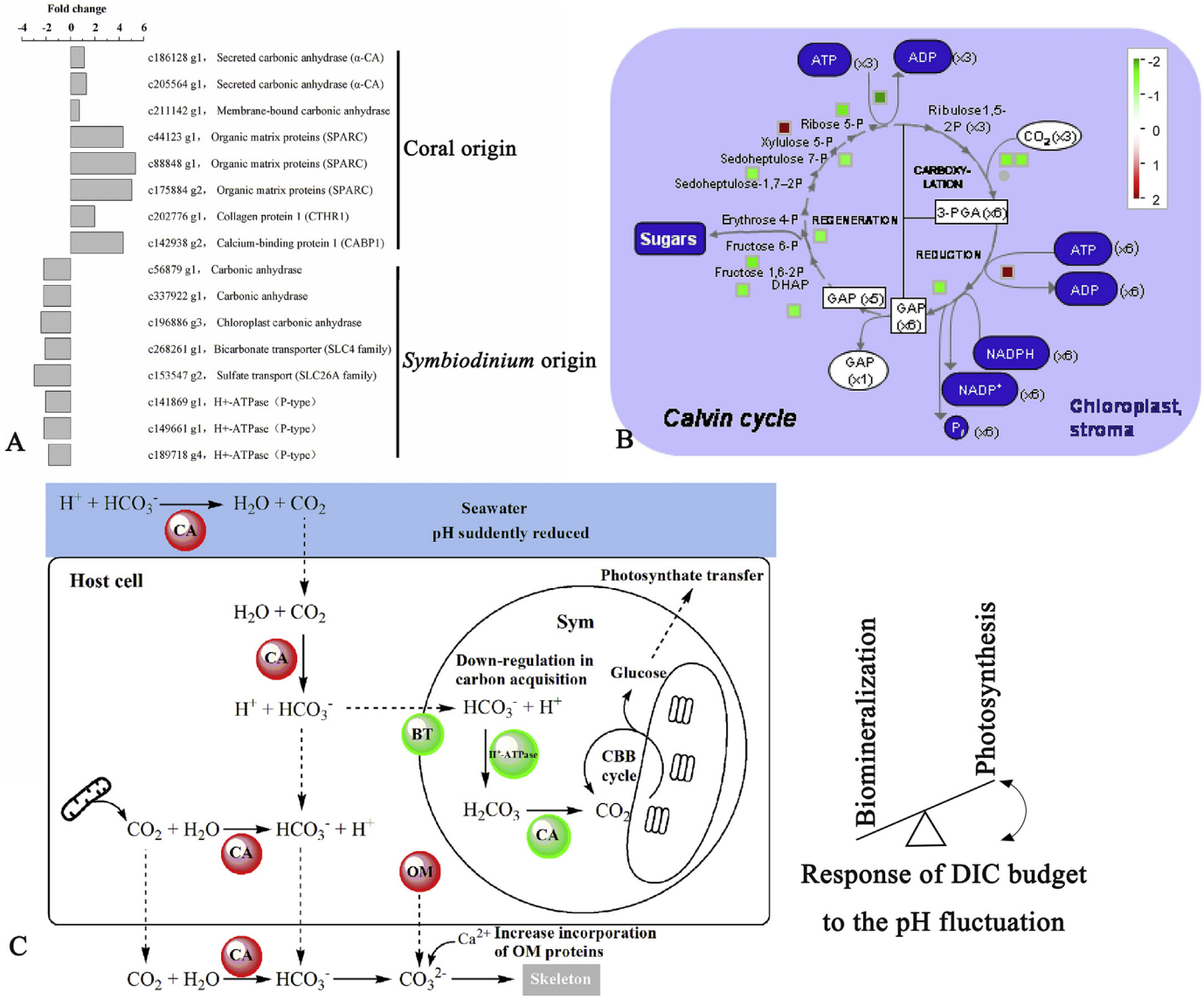

Fig. 3. Impacts of abrupt pH change on DIC flow pathway. (A) Overview of calcification and carbon uptake-related regulated genes of coral–algae symbiosis with differential expression changes in pH 7.60 vs pH 8.35. (B) Calvin cycle-related regulated genes of endosymbionts, with differential expression changes in pH 7.60 vs pH 8.35. (C) A schematic model summarizes the main possible effects of the external pH change on the carbon budget of the coral–algae symbiosis. Green and red blocks denote downregulated and upregulated genes, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the homeostasis status of the coral holobiont, in terms of its intracellular energy reserves and nutrition pool, should have roles in its ability to tolerate external stress.

3.3. Impacts of reduced pH on DIC availability pathway

One important finding was that the large coral origin genes coding for the extracellular secreted and membrane-bound carbonic anhydrases (α-CA, Fig. S2) (which were an inherent part of the inorganic carbon concentrating process (CCM) in corals and which support calcification and photosynthesis (Bertucci et al., 2011), organic matrix (OM) proteins, including acid-rich protein (SPARC) – namely, CTHR1 and CABP1 – that participated in calcium carbonate crystal formation [2]) were notably upregulated in the acute acidification exposure response. However, in turn, we observed a negative response of endosymbionts to low pH conditions. The *Symbiodinium*-origin CA, HCO_3^- transporter (BT) and other multifunctional Na^+ channels (SCL26 family) and H^+-ATPase genes showed an uncoordinated downregulation (Fig. 3A). Furthermore, too low pH almost totally repressed the expression of the genes that were related to the Calvin cycle (Fig. 3B). It was likely that the external pH reduction triggered a repression in photosynthetic CO_2 uptake and fixation pathways by downregulating the related transcript pool of the endosymbiont algae, which was bound to reduce carbon flux export to photosynthesis. Collectively, our results suggested that coral, of which CAs expressed at a high level may accelerate the conversion of DIC (or CCM induction) to the calcifying fluid, but limit the DIC cost for photosynthesis, the incorporation of OM proteins also increased simultaneously at the site of calcification. This process may help to facilitate increased rates of carbonate precipitation to cope with low pH exposure (Fig. 3C).

3.4. The transcript functional signatures involved in pH stress responses

Some noteworthy DEGs relevant to pH stress responses are listed in Table 2. Gene functional analysis revealed that DEGs from the acute acidification included significantly more genes associated with response

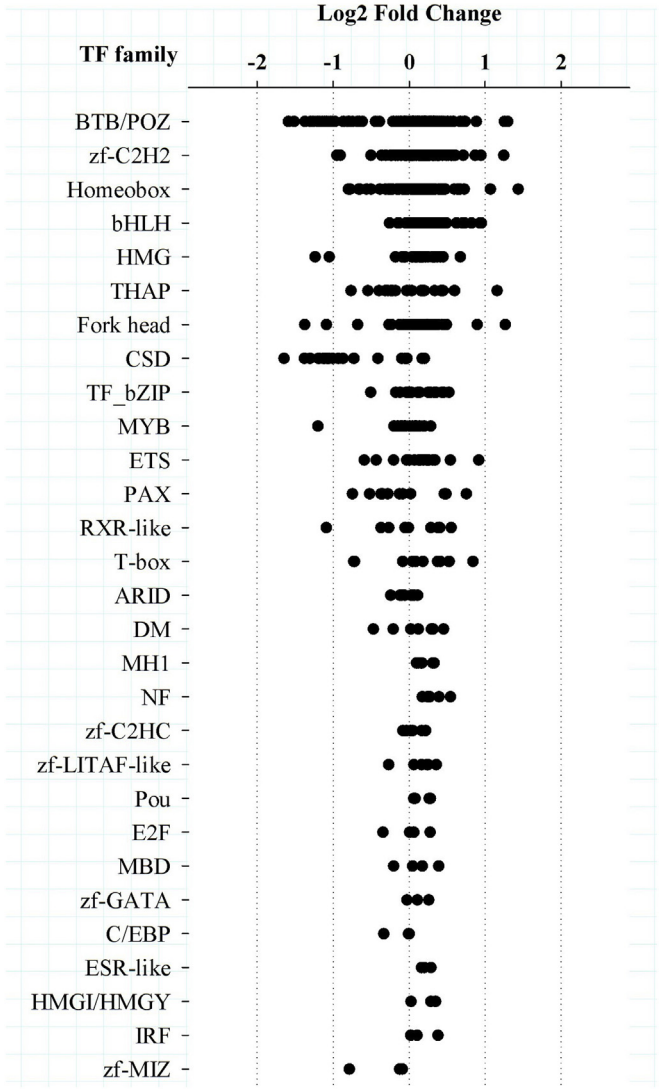

Fig. 4. Abrupt pH change influences the expression of transcription factor family genes in coral. The fold change (pH 7.60 vs pH 8.35) in expression for the transcription factor families.

to stress and protein damage than would be expected by chance. Examples from this category include heat shock proteins (HSP) and molecular chaperones (DNAJ), both of which were regulated intensely in the sudden external pH change. Particularly, the increase (> 10-fold) in the transcript level of two HSP-associated proteins (HSP70 and HS12B) in *G. fascicularis* under sudden CO_2 elevation again agrees with previous studies (Moya et al., 2015). Seven different protein degradation genes were upregulated in decreased pH; conversely, five proteolysis genes were downregulated in that treatment. Six genes in the categories of serine/threonine protein kinases, the magnitude of their expression changes, and their known roles in defense processes all play important roles in environmental sensing and adaptation associated with pH change. A set of genes (8 genes) that was associated with the transport and exchange of calcium and other ions was specifically regulated under the pH fluctuation and may be involved in maintaining cellular acid–base homeostasis (Stumpp et al., 2012). Additionally, Fig. S3 summarized the PPI-network of predicted associations for DEG-encoded proteins. The results indicated that the proteins DHRSX, PDXK, ODC1, ACLY, ENO1 and IDH1 were upregulated during the decreased pH exposure and were key in the interaction network; they were linked to ATP citrate lyase signaling, which provided an ability to effectively manage the greater energy metabolic cost by allocating ATP. However, five proteins, including PPIP5K1, SSRP1, WDHD1, RRM1 and GINS4, which were responsible for cell apoptosis, were downregulated under CO_2 elevation.

3.5. Negative regulation of CSD and BTB/POZ-mediated acid stress responses

In silico analyses against the animal TFDB 2.0 database were performed. A total of 684 transcription factors (TFs) and transcription coregulators (TCs) of the coral-derived transcriptome were identified and divided into 57 gene families, including BTB/POZ (125), zf-C2H2 (99), homeobox (93), Bhlh (53), HMG (36), and THAP (30), among others (Fig. S4). Of these, the differential expression pattern showed that the vast majority of transcription factor genes did not show strong expression changes in response to abrupt pH change, except for a limited number of CSD, forkhead, HMG, homeobox, BTB/POZ and zf-C2H2 genes (Fig. 4). Notably, we found an overall expression trend where the transcription factor genes associated with the CSD family (44%) and BTB/POZ family (12%) showed generally repressed transcription (log2-fold changes > 1.0) under the pH 7.60 treatment, implying that CSD and BTB/POZ could be very susceptible to acute pH events. Accumulating evidence has shown that the downregulation or dysfunction of the CSD and BTB/POZ proteins is linked directly or indirectly to metabolic adaptation, cell cycle and developmental disorders (Cho et al., 2012).

4. Discussion

Despite the extreme-pH environmental conditions and acute acidosis, stressors are a rare occurrence in nature, but some populations of upwelling, intertidal and estuarine corals might experience an extreme pH exposure for periods of up to several hours (**Rosser and Veron,** 2011). Here, our experimental design provides an opportunity to examine a theoretical exercise on the acute transcriptomic response of host and endosymbiont to an extreme pH/pCO_2 change event, and the possible schematic representation of mechanism is summarized in Fig. 5. The results suggest that additional metabolic costs for the acute acid adaptation response, such as increased rates of protein damage, DNA repair, ion transport, cellular apoptosis, maintenance of intracellular pH homeostasis and maintenance of stress defenses, collectively account for most of the energy use. Further, the allocation of energy resources and the nutritional pool for a greater expenditure of metabolic substrates may be a key basis for the coral holobiont response to additional energy – and nutrition – demanding environmental stressors. The discussion in this study focuses on the outstanding features of the potential effects of CO_2 elevation on energy-balance and the carbon budget of the coral–algae symbiosis.

Coincident with many shorter-term studies, marine calcifying organisms can increase rates of many biological processes over a short period, such as energy generation and calcification ability, in order to compensate for the negative effects of increased seawater acidity (Vidal-Dupiol et al., 2013). Our results indicate that corals will attempt to cope with changes in the seawater acid–base balance as an acute response, and a broad-scale upregulation of the secreted and membrane-bound CAs that may accelerate the conversion of DIC to the calcifying fluid (Bertucci et al., 2013); meanwhile, the increased incorporation of skeletal OM proteins will offer greater potential for enabling calcification with a sufficient carbonate saturation state under less favorable pH conditions. However, the upregulation of calcification and adaptive metabolism, potentially ameliorating some of the effects of increased acidity, comes at a substantial energy cost and is therefore unlikely to be sustainable in the long term (Wood et al., 2008). Severe or persistent acidosis could even lead to a loss of calcification adaptability, as shown in the study by Moya et al., (Moya et al., 2012), where 3 days of exposure of *Acropora millepora* to elevated levels of CO_2 led to a disruption in the levels of expression of CAs and many OMs implicated

Table 2
Significantly differentially expressed genes in response to stress are identified by the transcriptome experiment comparing gene expression between the pH 7.60 and pH 8.35. Common names are provided for the top BLAST hits in the Swiss-Prot database.

Gene ID	Gene	Description	Fold change	FDR
Protein degradation				
c126429_g2	CLPP2	ATP-dependent Clp protease proteolytic subunit 2	4.933	0.042
c288308_g1	SOHB	Probable protease SohB	4.086	0.017
c267868_g1	TMPS9	Transmembrane protease serine 9	3.471	0.05
c153818_g2	MA2B1	Lysosomal alpha-mannosidase	5.685	0.013
c315416_g1	MCA1	Metacaspase-1	10.366	0.012
c130977_g2	PSD7A	26S proteasome non-ATPase regulatory subunit 7 homolog A	10.816	0.002
c100395_g1	POMP	Proteasome maturation protein homolog	10.059	0.011
Ubiquitin-mediated				
c197689_g1	UBP25	Ubiquitin carboxyl-terminal hydrolase 25	−2.022	0.012
c147730_g1	UBIL	Ubiquitin-like protein	−1.856	0.046
c44266_g1	LUL3	Probable E3 ubiquitin-protein ligase LUL3	−2.405	0.041
c336775_g1	TRI18	E3 ubiquitin-protein ligase	−2.05	0.031
c131810_g1	UBP16	Ubiquitin carboxyl-terminal hydrolase 16	−2.028	0.041
Serine/threonine-protein kinase				
c37623_g1	AUR3	Serine/threonine-protein kinase Aurora-3	−2.169	0.027
c192719_g1	KPSH1	Serine/threonine-protein kinase H1	−1.861	0.047
c348850_g1	NEK4	Serine/threonine-protein kinase Nek4 = 2	9.595	0.039
c211179_g3	Y1743	Probable LRR receptor-like serine/threonine-protein kinase At1g74360	−2.039	0.033
c102369_g1	Y9871	Probable serine/threonine-protein kinase DDB_G0271682	−2.179	0.046
c95864_g2	PPT1	Serine/threonine-protein phosphatase T	5.149	0.006
Ion transport and regulation				
c208883_g1	SLC22AF	Solute carrier family 22 member 15 SLC22a15	−2.088	0.018
c165562_g2	SLC35E4	Solute carrier family 35 member E4 SLC35e4	10.063	0.025
c177181_g2	SLC44A3	Choline transporter-like protein 3 SLC44A3	9.714	0.027
c116981_g2	SLC38AB	Putative sodium-coupled neutral amino acid transporter 11 SLC38A11	9.866	0.028
c165860_g1		Ion transport protein	−2.3833	0.029
c51017_g1		Two pore potassium channel C	−2.7285	0.046
c108432_g1	HMA2	Cadmium/zinc-transporting ATPase HMA2	4.474	0.028
c175941_g1	MJ1226	Putative cation-transporting ATPase MJ1226	10.74	0.003
Heat shock proteins				
c197507_g7	HS12B	Heat shock 70 kDa protein 12B	10.299	0.046
c114043_g1	HSP70	Heat shock 70 kDa protein	10.592	0.009
c152362_g1	HSP20	HSP20	−1.832	0.033
Molecular chaperones				
c150023_g1	DNAJ	Chaperone protein DnaJ	−2.336	0.049
c37098_g1	DNJ10	Chaperone protein DnaJ	−2.157	0.029

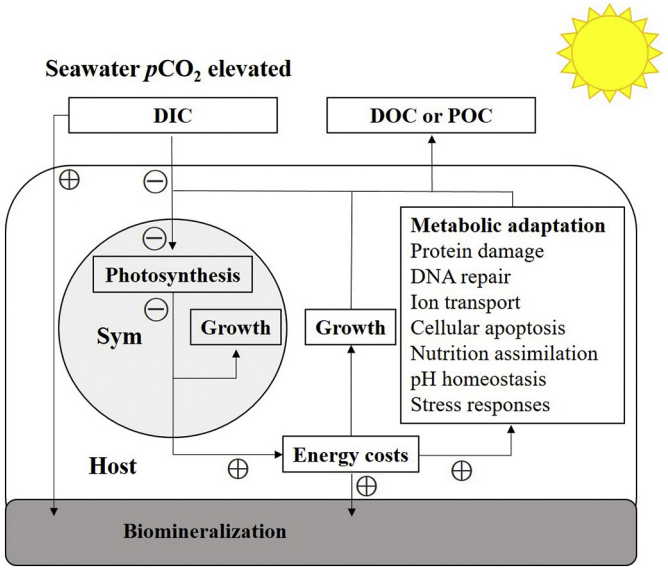

Fig. 5. Schematic model proposed for the main possible effects of abrupt pH change on the energy cycle of the coral–algae symbiosis. ⊕ and ⊖ indicate reactions or genes that are upregulated and downregulated in the metabolic pathways, respectively.

in skeleton deposition. We are beginning to understand that the ability for some coral species to undergo temporal dynamic biological control processes toward calcification to rapidly acclimate to stressors; however, a fundamental premise is that it occurs below the threshold for CO_2-induced bleaching (Berkelmans, 2002). After all, the species or genetic basis of the calcified corals vary in their ability to cope with acidification.

In coral-algae symbioses, photosynthesis and calcification do not occur independently (Bertucci et al., 2013); CCM plays a very important role in corals involved both in the regulation of calcification processes and in the supply of CO_2 for symbiont photosynthesis. Meanwhile, carbon uptake is mediated by bicarbonate transport, H^+-ATPase and CA in the endosymbionts, which is an effective strategy for DIC acquisition and the simultaneous enhancement of carbon fixation that enables *Symbiodinium* adaptation to the symbiotic life style. This limited study shows that the expression level of all the DIC uptake related genes in the endosymbionts are significantly downregulated in response to elevated pCO_2 and may lead to the reduced potential efficiency of carbon acquisition, causing the endosymbionts to rely simply on passive diffusion of CO_2 alone from the symbiosome membrane (Price et al., 2011). The implications are that the environmental pH conditions are capable of affecting expression of the specific DIC carriers of endosymbionts. Previous studies have suggested that the H^+-ATPase had no detectable expression in free-living *Symbiodinium*, and its expression level was totally dependent on the symbiotic state (Bertucci et al., 2009). Thereby, in this study, the fine expression regulation related to

carbon budget is possibly the result of the interaction changes between the host and the symbionts to adapt to the unusual pH situation. However, questions about the intrinsic adaption mechanism remain such as why high CO_2 induces CCM to drive DIC sources to coral calcification, while limiting the DIC supply to photosynthesis of its symbiont partners, and how is this affected by the environmental pH/ionic control.

Furthermore, the decreased levels of the genes encoding the dark reaction (CBB cycle) in response to the sudden high CO_2 dosing deems that the negative effect of high-CO_2 is highlighted by photoinhibition processes (Anthony et al., 2008). Repression of the CBB cycle could result in a carbon sink limitation and a dramatic decrease in the metabolic flux of photosynthesis; the negative results would also probably include a decrease in quantum yields of PSII fluorescence (ΔF/Fm´) and an increase in nonphotochemical quenching (*qN*), while decreases in gross photosynthesis would occur (Jones et al., 1998). Furthermore, a number of studies have clearly illustrated the importance of photoinhibition in exacerbating oxidative stress and damage to PSII, which may also trigger the onset of ROS and oxidative damage and is the proximal cause of coral bleaching (Wooldridge, 2009).

Indeed, high-CO_2 dosing has been previously shown to cause a further reduction in productivity to near zero in *Acropora* and crustose coralline algae (Anthony et al., 2008); the decreased capacity in autotrophic carbon under low pH are also reported for *Stylophora pistillata* (Tremblay et al., 2012). Tremblay has found that the amount of photosynthates translocated to the host were significantly higher under low pH (Tremblay et al., 2013). In symbionts, > 95% of photosynthates are exported to the host as an energy source. Particularly, when ocean acidification occurs, there is an increased energy demand of the coral host to maintain the metabolic adaptation, nutrient assimilation, calcification function and the ability to cope with stress-induced damage. The loss of photosynthetic production, therefore, severely diminishes the energy gains of corals and appears to be an unsustainable continuum in the 'coral-algae' energy cycle. Furthermore, the energy-costly disturbances would trigger a period of resource ill-balance and increase the risk of coral mortality (Anthony et al., 2009). Due to a lack of heterotrophic and autotrophic plasticity in coral, the continuous energy stepwise overdraft process can lead to organism energy insufficiency (Anthony and Fabricius, 2000), positively reinforcing the collapse of coral-algae endosymbiosis, leading to the expulsion of *Symbiodinium*, owing to persistent production loss once the energy budgets and availability of the holobiont fall below the level that triggers the onset of bleaching events (Wooldridge, 2010). Taken together, the results extend the global transcriptomic changes to consider the possibility that acute acidification initially triggers a breakdown in the stability of the energy cycle, resulting in a dysfunction of the coral-algal symbiosis (Lin et al., 2017a), and limits the ability to maintain a continuous mutualistic benefit between the coral and its endosymbiotic partners; this is especially problematic for a sensitive coral under abrupt pH reduction or high pCO_2 stress.

5. Conclusion

This transcriptome study provides a number of new insights regarding the global regulation of coral host-derived genes and endosymbiotic *Symbiodinium*-derived genes by a sudden pH or CO_2 change. The results show that the endosymbiont demonstrates a significant suppression of molecular pathways related to carbon acquisition and fixation, suggesting the photosynthetic dysfunction in algal symbionts exposed to elevated pCO_2, which would interference with the energy cycle in coral-algal symbiosis. The transcriptomic would also suggest the coral is still capable of calcifying in response to low pH, but would experience a series of negative effects on their energy dynamics, thereby suggesting enhanced energy costs for the coral metabolic adaptation. Overall, the data presented herein extend our understanding of the adaptation biology of symbiotic corals coping to potentially frequent environmental fluctuations or extreme climate changes in the near future.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.margen.2018.08.006.

Data archiving statement

The transcriptome assembly of *G. fascicularis* and its *Symbiodinium* endosymbionts, as well as RNA sequencing data (unigenes), were deposited in the NCBI Transcriptome Shotgun Assembly (TSA) under accession number GFAZ00000000. The raw data for the pH treatments are publicly available at NCBI SRA (accession number SRX2612345).

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Author contributions

ZYL and LYW conducted the experiments. ZYL analyzed the data. ZYL wrote the manuscript. ZYL, MLC and JMC conceived of the study. All authors reviewed and commented on the manuscript.

Competing financial interests

The authors declare no competing financial interests.

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